



Associate Editor: D. Shugar

p38 Mitogen-Activated Protein Kinase Inhibitors— Mechanisms and Therapeutic Potentials

John C. Lee,* Shouki Kassis, Sanjay Kumar, Alison Badger and Jerry L. Adams

SMITHKLINE BEECHAM PHARMACEUTICALS, KING OF PRUSSIA, PA 19406, USA

ABSTRACT. The pyridinylimidazole compounds, exemplified by SB 203580, originally were prepared as inflammatory cytokine synthesis inhibitors. Subsequently, the compounds were found to be selective inhibitors for p38 mitogen-activated protein kinase (MAPK), a member of the MAPK family. SB 203580 inhibits the catalytic activity of p38 MAPK by competitive binding in the ATP pocket. Four homologues of p38 MAPK have been identified to date, and interestingly, their biochemical properties and their respective sensitivities to the inhibitors are distinct. X-ray crystallographic analysis of p38-inhibitor complexes reinforces the observations made from site-directed mutagenesis studies, thereby providing a molecular basis for understanding the kinase selectivity of these inhibitors. The p38 MAPK inhibitors are efficacious in several disease models, including inflammation, arthritis and other joint diseases, septic shock, and myocardial injury. *PHARMACOL. THER.* 82(2–3):389–397, 1999. © 1999 Elsevier Science Inc. All rights reserved.

KEY WORDS. p38 MAP kinase, inhibitors, pyridinylimidazole, mechanism of action, inflammatory disease, cytokine signaling.

CONTENTS

1. INTRODUCTION	389	6. STRUCTURAL BASIS OF SELECTIVITY EXPLORED THROUGH MUTAGENESIS	393
2. P38 MITOGEN-ACTIVATED PROTEIN KINASE FAMILY MEMBERS AND SUBSTRATES	390	7. X-RAY DATA	394
3. INHIBITORS OF P38 MITOGEN-ACTIVATED PROTEIN KINASES	392	8. PHARMACOLOGY OF PYRIDINYLIMIDAZOLE P38 MITOGEN-ACTIVATED PROTEIN KINASE INHIBITORS	394
4. KINASE INHIBITION AND BINDING	392	9. CONCLUSION AND PERSPECTIVES	395
5. KINASE SELECTIVITY	393	ACKNOWLEDGEMENTS	395
		REFERENCES	395

ABBREVIATIONS. AA, adjuvant arthritis; ATF, activating transcription factor; CHOP, C/EBP homologous protein; CREB, cyclic AMP response-element binding protein; eIF, eukaryotic initiation factor; ERK, extracellular-signal regulated kinase; GST, glutathione S-transferase; IL, interleukin; JNK, c-Jun N-terminus kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MAPKAP, mitogen-activated protein kinase-activated protein; MEK, myocyte enhancer factor; MKK, mitogen-activated protein kinase; MNK, mitogen-activated protein kinase-interacting kinase; MSK1, mitogen- and stress-activated protein kinase 1; OVA, ovalbumin; SAPK, stress-activated protein kinase; SAR, structure-activity relationship; TGF, tumor growth factor; TNF, tumor necrosis factor.

1. INTRODUCTION

We have been interested in defining the molecular mechanisms regulating cytokine biosynthesis. A series of low-molecular weight compounds were found to inhibit lipopolysaccharide (LPS)-stimulated interleukin (IL)-1 and tumor necrosis factor (TNF) production in human monocytes (Lee *et al.*, 1988; 1989). One representative compound, SK&F 86002, when tested at its IC_{50} for cytokine inhibition, had no appreciable effect on DNA, RNA, or protein synthesis (Lee *et al.*, 1990, 1993). Furthermore, its inhibitory activity on IL-1 production was observed with a number of different stimuli and target cells. Optimal inhibition was observed when the cells were pretreated or treated early (<2 hr) in the induction phase of IL-1 expression. Be-

sides IL-1, other cytokines, such as TNF- α , IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor, but not granulocyte colony-stimulating factor or IL-1 receptor antagonists, were inhibited (Lee *et al.*, 1990). Western blot analysis confirmed that the intracellular levels of IL-1 and TNF were significantly reduced in LPS-treated human monocytes and were not paralleled by similar changes in the respective mRNA (Young *et al.*, 1993). These studies establish a profile of cytokine inhibitory properties for SK&F 86002 that is uniquely different from the known anti-inflammatory agents. While mechanistic studies suggest that cytokine synthesis is regulated at the translational level, a full understanding of the compound's action required identification of the molecular target of the pyridinylimidazoles. Therefore, radiolabeled and radiophotoaffinity ligands were prepared to help identify, isolate, and determine the peptide sequence of the molecular target and clone its cDNA.

*Corresponding author's address: Department of Bone and Cartilage Biology, SmithKline Beecham Pharmaceuticals, UJW2109, 709 Swedeland Road, King of Prussia, PA 19406, USA.

[³H]SB 202190 was synthesized and its uptake in THP.1 cells, a human monocytic cell line, was measured. When treated with LPS, these cells show an identical pattern of cytokine production and sensitivity to drug inhibition to that observed in human peripheral blood monocytes. Uptake of the radioligand is time- and temperature-dependent, saturable, and competitive with unlabelled SB 202190, but not with an inactive analog (Lee et al., 1994). These results suggest that a compound binding molecule exists in THP.1 cell lysates. A binding assay subsequently has been configured to quantitate the binding of [³H]SB 202190 to the cytosolic fraction of THP.1 cells. The binding is specific, time-dependent, reversible, and of high affinity ($K_d \approx 50$ nM) (Lee et al., 1994). Importantly, when many structural analogs were examined, a high degree of correlation was established between cytokine biosynthesis inhibition and competition in the binding assay. This confirmed that the binding activity was related to cytokine inhibition. Size exclusion chromatography of cell lysates and sensitivity to proteolysis indicated that the molecular target was a protein of ca. 40 kDa. However, we were unable to purify the target more than ~20-fold, as determined by the binding assay, and attempts to affinity purify the protein using compound-bound resin were similarly ineffective. Therefore, the radiophotoaffinity analog [¹²⁵I]SB 206718 was synthesized to aid in the purification of the drug-binding protein. Remarkably, the radiophotoaffinity ligand crosslinked to a single 43 kDa protein in partially purified cell lysates. Crosslinking of SB 206718 was blocked by unlabelled active, but not inactive, compounds in the same rank order of potency as seen in the binding assay (Lee et al., 1994).

Two unique peptide sequences were obtained by tryptic and chemical digestion and were used to make reverse-translated synthetic oligonucleotides. These were used as probes to screen a granulocyte-macrophage colony-stimulating factor-stimulated human monocyte library, and two cDNAs containing an open reading frame encoding a protein of 360 amino acids were identified. They were identical in sequence except for an internal region of 75 bp, which shared only 43% identity at the nucleotide and protein level. The two protein products were named CSBP 1 and 2. Expression of these two proteins in *Escherichia coli* and yeast showed that they both could bind to the radioactive and radiophotoaffinity compounds (Lee et al., 1994; Kumar et al., 1995). Based on the primary amino acid sequence and a comparison to known proteins in the GENBANK database, the proteins were found to be novel members of the mitogen-activated protein kinase (MAPK) family. All of the MAPKs are characterized by an activation loop containing the sequence Thr-Xaa-Tyr, which is phosphorylated on both Thr and Tyr by a MAP kinase kinase (MKK) in response to extracellular stimuli (Cobb and Goldsmith, 1995). This phosphorylation results in activation of the kinase activity of the MAPK.

There are at least three families of MAPKs that differ in the sequence and size of the activation loop: the extracellular signal-regulated kinases (ERKs) have a TEY motif; the

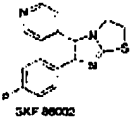
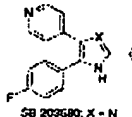
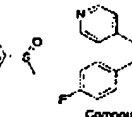
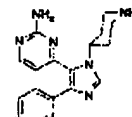
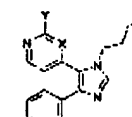
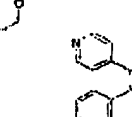
c-Jun N-terminus kinases (JNKs) or stress-activated protein kinases (SAPKs), which have a TPY motif; and the p38 family, which has a TGY motif. The ERKs are stimulated by mitogens and several 7-transmembrane receptor agonists (Cobb and Goldsmith, 1995), resulting in the sequential activation of Ras, Raf, MKK1 or 2, and ERK, which in turn phosphorylates transcription factors such as Elk1 and STAT1, thereby stimulating their transcriptional activity (Gille et al., 1992; Wen et al., 1995). Both the JNKs and p38 MAPKs are activated by various stress stimuli, including UV, heat, chemical or osmotic shock, IL-1, TNF, and endotoxin (Derijard et al., 1994; Kyriakis et al., 1994; Han et al., 1994; Rouse et al., 1994; Freshney et al., 1994; Lee et al., 1994; Kracht et al., 1994; Raingeaud et al., 1995). However, more recent studies indicate some differences in the MKKs that activate these two MAPKs, since p38 MAPK is activated by MKK3 and MKK6, whereas MKK4 and MKK7 activate JNK (Sanchez et al., 1994; Derijard et al., 1995; Lin et al., 1995; Han et al., 1996; Raingeaud et al., 1996).

2. p38 MITOGEN-ACTIVATED PROTEIN KINASE FAMILY MEMBERS AND SUBSTRATES

Subsequent to the identification and cloning of p38 MAPK (Han et al., 1994; Lee et al., 1994), at least four distinct homologues, standardized in the nomenclature as p38 α , β , γ (SAPK3), and δ (SAPK4), have been identified (Jiang et al., 1997; Kumar et al., 1997; Stein et al., 1997; Mertens et al., 1996; Goedert et al., 1997), either by homology cloning or by searching the expressed sequence tag database using sequences specific to p38 MAPK. The p38 β homologue was described by Jiang et al. (1997) as having an 8 amino acid insertion. Subsequently, the correct sequence lacking the insertion was reported in two studies (Stein et al., 1997; Kumar et al., 1997). A common feature of these homologues is the presence of a 12 amino acid activation loop comprising the "TGY" motif. These kinases are ~60–70% identical to each other. Most of these kinases are widely expressed, except p38 γ , which shows the highest expression in skeletal muscle. Among the upstream kinases, MKK3 and MKK6 activate one or more of p38 isoforms. MKK3 has been shown to selectively activate p38 α and p38 γ (Enslen et al., 1998), while MKK6 activates all four isoforms (Goedert et al., 1997; Cuenda et al., 1997). MKK4 activates both p38 and JNK (Jiang et al., 1997). While a few physiological substrates of p38 α MAPK have been identified, the exact physiological *in vivo* substrates for the other isoforms are unknown. *In vitro*, several proteins act as substrates for p38 α or other p38 isoforms (Kumar et al., 1997). Myelin basic protein, a generic substrate for many protein kinases, serves well as a substrate for all p38 isoforms.

MAPK-activated protein (MAPKAP) K2 and MAPKAP K3 have been identified as physiological substrates for p38 MAPK, since treatment of cells with SB 203580 (Table 1) inhibited the activation of MAPKAP K2 kinase (Cuenda et al., 1995). MAPKAP K3 was identified in a yeast two hybrid screen using p38 MAPK as bait (McLaughlin et al.,

TABLE 1. Structures of p38 MAP Kinase Inhibitors

		
SKF 86002	SB 203580; X = N L-167307, X = CH	Compound 1
		
SB 220025	SB 210313; X = CH, Y = H SB 216385; X = N, Y = NO ₂	RWJ 68354

Compound	p38 (IC ₅₀ , nM)	Compound	p38 (IC ₅₀ , nM)
SKF 86002	1500	SB 220025	19
SB 203580	48	SB 210313	1300
L-167307	5.0	SB 216385	480
Compound 1	83	RWJ 68354	9.0

1996). MAPKAP K2/3 is activated rather poorly by p38 γ and p38 δ (Cuenda *et al.*, 1997; Kumar *et al.*, 1997). Activated MAPKAP K2/3 phosphorylates the small heat-shock protein 25/27, which may be involved in cytoskeletal reorganization. p38 β has been proposed to phosphorylate activating transcription factor (ATF)2 with higher efficiency than p38 α (Jiang *et al.*, 1997; Stein *et al.*, 1997), while others have shown that all four isoforms phosphorylate ATF2, as well as Elk1 and SAP1, with equal efficiency (Goedert *et al.*, 1997; Cuenda *et al.*, 1997; Kumar *et al.*, 1997).

Following phosphorylation of MAPKAP kinase-2, nuclear p38 is exported to the cytoplasm in a complex with MAPKAP kinase-2. The cytoplasm translocation of MAPKAP kinase-2 requires phosphorylation by p38 without a requirement for MAPKAP kinase-2 activity (Ben-Levy *et al.*, 1998). MAPKAP kinase-2 serves both as an effector of p38 by phosphorylating substrates and as a determinant of cellular localization of p38. Nuclear export of p38 and MAPKAP kinase-2 may permit them to phosphorylate cytoplasmic substrates, such as eukaryotic initiation factor (eIF)-4E and PHAS-1.

p38 Regulated/activated kinase is a 471-residue kinase with 20–30% sequence identity to other known kinase substrates. The kinase can be activated in response to cellular stress and pro-inflammatory cytokines, and is regulated by p38 α and p38 β and phosphorylates Hsp27 *in vitro* and *in vivo* (New *et al.*, 1998). A recent study (Preville *et al.*, 1998) used a nonphosphorylatable Hsp25 to define the physiological role of Ser15 and/or 86 phosphorylation, which has been suggested to interfere with hyperaggregation of the protein involved in the cytoprotective effect against oxidative stress.

Mitogen- and SAPK1 (MSK1) is a protein-serine/threonine kinase that contains two kinase domains in a single polypeptide (Deak *et al.*, 1998). Activation of MSK1 by

peptide growth factors/phorbol esters and environmental stimulation are blocked by the MKK/MEK inhibitor PD 98059 and the p38 inhibitor SB 203580, respectively. In HeLa and PC 12 cells, both PD 98059 and SB 203580 are required to block MSK1 activation by TNF, nerve growth factor, and fibroblast growth factor, which is consistent with the fact that these agonists activate both the ERK and p38 MAPK cascades. The pharmacological inhibition profile and agonist-induced activation of cyclic AMP response-element binding protein (CREB) and ATF1 mirror MSK1 activation, suggesting that MSK1 may mediate growth factor and stress-induced activation of CREB (Deak *et al.*, 1998). MAPK interacting kinase 1 and 2 (MNK 1/2) are protein serine/threonine kinases identified to physically associate with MAPKs (Waskiewicz *et al.*, 1997; Fukunaga and Hunter, 1997). MNK1 and 2 are structurally similar to MAPKAP K2/3 and p90^{RSK}. MNK1 binds tightly to ERK1/2 and p38, whereas MNK2 binds only ERK1/2. Both ERKs and p38 phosphorylate and activate MNK1 in response to peptide growth factors/phorbol esters and environmental stress, and the activation by these stimuli is differentially inhibited by PD98059 and SB 202190 (Fukunaga and Hunter, 1997). *In vitro*-activated MNK1 phosphorylates eIF-4E at the physiologically relevant site, Ser209 (Waskiewicz *et al.*, 1997; X. Wang *et al.*, 1998).

While a number of transcription factors are phosphorylated *in vitro* by p38, the physiological relevance of this phosphorylation is unknown. CHOP (C/EBP homologous protein) is a member of the C/EBP family of transcription factors that accumulates under conditions of stress and mediates the effect of cellular stress on growth and differentiation. In response to stress, CHOP undergoes phosphorylation on adjacent serine residues, 78 and 81, which is blocked by SB 203580. *In vitro*, CHOP is phosphorylated on serine residues by p38 MAPK, and phosphorylation positively correlates with its transcription activity (Wang and Ron, 1996).

Myocyte enhancer factor (MEF)2C was identified in a yeast two hybrid screen using p38 MAPK as bait (Han *et al.*, 1997). In monocytes, LPS enhances the transcriptional activity of MEF2C through p38-catalysed phosphorylation. Activation of MEF2C results in increased c-jun transcription, suggesting that p38 may influence host defense and inflammation by regulating c-Jun production. ATF2 is efficiently phosphorylated by all four isoforms of p38 and by JNK1 and 2 *in vitro*. While it is an excellent *in vitro* substrate for p38 and other isoforms, it is not clear if ATF2 is a physiological substrate of p38 α since the transcriptional activation or phosphorylation-mediated mobility shift in ATF2 has not been correlated yet with pharmacological inhibition of p38 MAPK. Elk1 and SAP1 are also phosphorylated efficiently. As with ATF2, it remains to be elucidated whether they are true physiological substrates *in vivo*. Recently, stathmin, a cytoplasmic protein that is linked to the regulation of microtubule dynamics and is a substrate for several intracellular signaling kinases, was also identified to be a substrate for p38 δ in a solution kinase assay. Osmotic stress-activated p38 δ has been shown to phosphorylate

stathmin on Ser25 and Ser38 *in vitro* and in cells (Parker et al., 1998).

3. INHIBITORS OF p38 MITOGEN-ACTIVATED PROTEIN KINASES

The p38 kinase inhibitors described thus far contain a common set of structural features. The important elements of the pharmacophore derived from a series of several tri- and tetra-substituted imidazole inhibitors of p38 have been described (Gallagher et al., 1997). In summary, all imidazole-based p38 inhibitors contain the elements of a 4-aryl-5-(pyridin-4-yl)imidazole. Further substitution of the imidazole at N-1 adjacent to the azaheteroaryl (SKF 86002 and SB 220025) or at C-2 is allowed (SB 203580), while substitution of the imidazole nitrogen adjacent to the aryl group is not. With the recent publication of several alternatives to the central imidazole, the scope of known p38 inhibitors has been broadened considerably (Hanson, 1997; Henry et al., 1998; de Laszlo et al., 1998).

de Laszlo et al. (1998) described the synthesis of regioisomeric furan, pyrrole (L-167307) and pyrazolinone (Compound 1) triaryls. In each case, one regioisomer is preferred, and the pyrrole is the best of the three imidazole replacements. The structure-activity relationship (SAR) derived from optimization of L-167307 paralleled the trend observed by Gallagher et al. (1997), suggesting a similar binding mode. The 1,4,5-substitution pattern of the core imidazole has also been explored (SB 210313) (Boehm et al., 1996). The SAR for this series has been extended to the examination of substituted pyrimidines as replacements for the 4-pyridyl (SB 216385) (Adams et al., 1998). Significantly, several of these pyrimidines demonstrate improved *in vitro* and *in vivo* activity relative to the 4-pyridyl compound. The activity and selectivity of a more potent pyrimidinyl imidazole (SB 220025) in an *in vivo* model of angiogenesis has been reported recently (Jackson et al., 1998). The p38 activity of representative inhibitors is detailed in Table 1.

4. KINASE INHIBITION AND BINDING

A sensitive kinase assay was developed for p38 MAPK using the T669 peptide substrate derived from the intracellular domain of the epidermal growth factor receptor (Young et al., 1997). Double reciprocal plot of initial velocity versus ATP concentration indicates that the value of K_m [ATP] in the absence of inhibitor is 170 μ M (Young et al., 1997). Initial velocity determinations performed in the presence of several concentrations of SB 203580 demonstrates that the inhibitor competes with ATP and has a K_i value of 21. Similar observations have been reported by others (LoGrasso et al., 1997; Frantz et al., 1998). The K_m [ATP] value observed using the T669 peptide substrate is about 7-fold higher than that reported using an ATF2-glutathione S-transferase (GST) fusion protein as the substrate (Frantz et al., 1998). The difference in the values of apparent K_m is likely to be due to the use of different phosphate acceptors, and may re-

fect the influence of a protein substrate on ATP binding affinity. In this regard, LoGrasso et al. (1997) observed that the kinetic reaction of p38 MAPK proceeds by a rapid-equilibrium ordered sequential mechanism with the ATF2-GST fusion protein substrate binding before ATP, and that binding of one substrate affects the binding of the other. This ordered sequential mechanism is unique for p38 MAPK as compared with cAMP-dependent protein kinase (Cook et al., 1982) and most tyrosine kinases (Boemer et al., 1995; Posner et al., 1992), and may explain the different K_m [ATP] values observed with T669 and ATF2-GST as phosphate acceptors.

Additional biophysical experiments described by Young and co-workers (1997) on the unactivated kinase offer useful insights into the mechanistic details of inhibitor binding. The binding of SB 203580 to *E. coli*-expressed unphosphorylated p38 has been determined by microcalorimetry. SB 203580 binds to p38 with \sim 1:1 stoichiometry and a K_D of 15 nM and a ΔH of -12 kcal at 30°C. In accordance with the ATP competitive inhibition kinetics, no binding of SB 203580 was observed when unactivated p38 was reacted with sulfonylbenzoyl adenosine, a covalent ATP-site inhibitor of kinases. Although *E. coli*-expressed p38 has no detectable protein kinase activity, the protein has intrinsic ATPase activity in the absence of substrate (k_{cat} 0.006 s $^{-1}$ and ATP K_m of 9.6 mM). The ATPase activity was completely inhibited by a 1-mol equivalent of SB 203580, $K_i \leq 100$ nM. Hence, SB 203580 potently inhibits the enzymatic activity of both the activated and unactivated forms of p38 MAPK.

Because of the high intracellular concentration of ATP, some 100-fold greater than the K_m , ATP competitive kinase inhibitors should demonstrate significantly attenuated activity in intact cells. However, the pyridinylimidazole inhibitors demonstrate equivalent potency in recombinant enzyme and cellular assays. Two models have been advanced to explain the lack of attenuation of activity in cellular systems (Frantz et al., 1998). The first model proposes that the lifetime of the activated enzyme is sufficiently short that the inactivation rate of activated-inhibitor complex by phosphatases is greater than the formation of the activated enzyme-ATP complex. If this were to occur, the inhibitor would behave as if it was noncompetitive with respect to ATP. In support of this proposal is the poor binding affinity of ATP to the unactivated p38 (K_m 9.6 mM) (Young et al., 1997) and the observation that effective competition by ATP with the pyridinylimidazoles is only seen with the activated kinase (Frantz et al., 1998). One caveat for this mechanism is that it assumes that the binding of protein substrates, which *in vivo* may exist as a complex or pre-associate with p38 prior to activation, does not substantially affect the K_m of ATP. Although this may be the case for unactivated p38, it clearly does not apply to activated p38, where binding of the protein substrate greatly increases the affinity of ATP binding. Measurement of the affinity of the enzyme-substrate complex for ATP, as well as measurements of the lifetimes of activated enzyme inhibitor

and activated enzyme-substrate-inhibitor complexes, will be required to assess the potential merit of this model.

Binding of pyridinylimidazole to p38 does not prevent its activation by p38 MKKs in cells. When HeLa cells are pre-treated with SB 203580 prior to addition of 0.4-mM sorbitol, activation of the downstream *in vivo* substrate of p38, MAPKAP kinase-2, as measured by *in vitro* phosphorylation of Hsp27, is inhibited. However, *in vitro* kinase assay of immunoprecipitated p38 from cells treated identically and washed free of the compound shows that SB 203580 has no inhibitory effect on the p38 kinase activity; whereas if SB 203580 is added back during the *in vitro* kinase reaction, inhibition of p38 activity is evident. Furthermore, SB 203580 has no effect on tyrosine phosphorylation of p38 MAPK when harvested directly from the cells without *in vitro* incubation. This and other data strongly indicate that SB 203580 binding does not prevent activation of p38 MAPK, which is in contrast with that reported by Frantz *et al.* (1998), which suggests that in LPS-stimulated THP-1 cells, the compound inhibits p38 activation as well. In another report, SB 203580 also fails to block activation of p38 in arsite-activated 293T cells, while it blocks the export of nuclear p38 to the cytoplasm (Ben-Levy *et al.*, 1998).

5. KINASE SELECTIVITY

A high level of selectivity for inhibition of p38 versus ~30 protein kinases has been reported for SB 203580 and is shown in Table 2 (Cuenda *et al.*, 1995; Young *et al.*, 1997; Kumar *et al.*, 1997; de Laszlo *et al.*, 1998; Evers *et al.*, 1998; S. Kassiss *et al.*, unpublished). Selectivity ratios based on IC₅₀s often exceed 1000. Whereas SB 203580 is equipotent against p38α and p38β, other closely related MAPKs, such

as p38γ, p38δ, JNK1, and Erk2, are not or are weakly inhibited. The most potent inhibition reported for non-p38 kinases by SB 203580 are c-Raf (IC₅₀ 360 nM) and JNK2β1 (IC₅₀ 280 nM). SB 203580 does not appear to be unique in its kinase selectivity profile, as selective inhibition of p38 kinase has been reported for several structurally related compounds (Jackson *et al.*, 1998; de Laszlo *et al.*, 1998).

6. STRUCTURAL BASIS OF SELECTIVITY EXPLORED THROUGH MUTAGENESIS

Mutagenesis studies have been used to explore the basis for the selective inhibition of p38 MAPK by the pyridinylimidazoles. Residues in the ATP pocket have been individually mutagenized and examined for their effects on kinase activity and p38 binding and/or inhibition (Young *et al.*, 1997). The data from these experiments are in agreement with subsequent X-ray crystallographic findings that the pyridinylimidazoles and ATP occupy different, although overlapping, regions of the ATP pocket. Based upon the X-ray crystallographic data implicating Thr106 as a key residue mediating selectivity, additional mutagenesis studies have focused on Thr106 and two adjacent residues at the back of the ATP pocket (Gum *et al.*, 1998). These same three residues are found in p38β, the closest homologue to p38α, but are not found in the more distant homologues p38γ and p38δ (Kumar *et al.*, 1997; Goedert *et al.*, 1997; Cuenda *et al.*, 1997). p38α and p38β are inhibited by SB 203580 and SB 202190, with IC₅₀s of 0.092 μM and 1 μM, respectively. p38γ and p38δ are not inhibited by either SB 203580 or SB 202190 at concentrations up to 50 μM. When these three residues in p38α (Thr106, His107, Leu108) are changed to the Met-Pro-Phe present in p38γ and p38δ, the enzyme activity is no longer inhibited by SB 203580 (Gum *et al.*, 1998). A single change of Thr106 to Met results in a 10-fold reduction in the sensitivity to SB 203580. In contrast, introduction of Thr-His-Leu into p38γ and p38δ, or the more distantly related JNK1, is sufficient to render these kinases sensitive to SB 203580. In these same three kinases, the single change to Thr106 led to an increase in sensitivity. Interestingly, the single change to or from Thr106 appears to be all that is required to obtain complete changes in sensitivity to SB 203580 when these enzymes are expressed in bacteria or yeast, which suggests that the other two residues may interact with other proteins in mammalian cells (Gum *et al.*, 1998; Evers *et al.*, 1998).

Mutagenesis studies by Evers *et al.* (1998) have explored the sensitivity of p38γ to mutations at position 106 (p38α equivalent). Native protein (Met106, IC₅₀ > 100 μM) was insensitive to SB 203580, as were all mutants having residues bulkier than threonine. Mutants having a threonine (IC₅₀ 0.3 μM) were sensitive to SB 203580, while smaller residues (alanine, IC₅₀ 0.01 μM) showed a further enhancement in SB 203580 sensitivity. Taken together, these data identify Thr106 as a key residue responsible for forming the 4-fluorophenyl specificity pocket and are fully consistent with the X-ray structural data. Thus, Evers *et al.* proposed

TABLE 2. The Sensitivity Profile among Protein Kinases to SB 203580

Kinase	Equivalent residue	SB 203580 (IC ₅₀ , nM)	Reference
p38α	T	48	Young <i>et al.</i> , 1997
p38β	T	50	Kumar <i>et al.</i> , 1997
p38γ	M	>10,000	Kumar <i>et al.</i> , 1997
p38δ	M	>10,000	Kumar <i>et al.</i> , 1997
JNK1	M	~5000	de Laszlo <i>et al.</i> , 1998
JNK2β1	M	280	de Laszlo <i>et al.</i> , 1998
JNK2α2	M	1900	de Laszlo <i>et al.</i> , 1998
ERK2	Q	>100,000	Cuenda <i>et al.</i> , 1995
Mek-1	M	61,000	Cuenda <i>et al.</i> , 1995
Cdc2	F	>50,000	Cuenda <i>et al.</i> , 1995
PKA	M	83,000	S. Kassiss, unpublished
PKC-β2	M	>50,000	S. Kassiss, unpublished
TGF-β1	S	20,000	Evers <i>et al.</i> , 1998
TGF-β11	T	40,000	Evers <i>et al.</i> , 1998
c-Raf	T	360	de Laszlo <i>et al.</i> , 1998
MAPKAP2	M	>10,000	Cuenda <i>et al.</i> , 1995
ZAP70	M	>20,000	de Laszlo <i>et al.</i> , 1998
LCK	T	20,000	Evers <i>et al.</i> , 1998
EGFR	T	10,000	S. Kassiss, unpublished

EGFR, epidermal growth factor receptor; PKA, protein kinase A; PKC, protein kinase C.

the broader application of the residue size at position 106 as a diagnostic to predict the sensitivity of any protein kinase to SB 203580. One such prediction, that tumor growth factor (TGF)- β R1 kinase would be sensitive, has proven to be true, although the potency of SB 203580 inhibition is two orders of magnitude less than its effect on p38 α *per se* (Eyers *et al.*, 1998). The selectivity data in Table 2 allow one to gauge the generality of Thr106 as a diagnostic feature. Although these data are supportive of the proposed diagnostic importance of Thr106, the varied conditions of the enzyme assays and failure to test at sufficiently high concentrations weakens the correlation. Nonetheless, the potent inhibition of c-Raf and weaker inhibition of TGF- β and tyrosine kinases are consistent with the proposed general role of the Thr106 residue for formation of the fluorophenyl binding pocket.

7. X-RAY DATA

Reports from two laboratories describe the X-ray crystallographic structure of native unactivated p38 α (Wilson *et al.*, 1996; Z. Wang *et al.*, 1997). More recently, multiple structures of pyridinylimidazole p38 complexes have become available (Li *et al.*, 1996; Tong *et al.*, 1997; Wilson *et al.*, 1997; Z. L. Wang *et al.*, 1998). The binding interactions defined by these structures are in good agreement with the published SAR for this class of inhibitors (Gallagher *et al.*, 1997). Important features observed in all of these structures are (1) the formation of a 4-fluorophenyl binding pocket behind and orthogonal to the site normally occupied by the adenine ring of ATP, (2) a well-formed hydrogen bond between the 4-pyridyl nitrogen and the amide N-H of Met109, and (3) H-bond-like interaction between Lys53 and the unalkylated imidazole N-H (Fig. 1).

The interaction between the 4-pyridyl group and the N-H of Met109 is analogous to the H-bond, which is seen for the N-1 adenine of ATP in all available kinase crystal structures. Moreover, this hydrogen bond acceptor-donor pair occurs in all inhibitor-kinase crystal structures. The interaction of the imidazole N-H with Lys53 is not a conserved feature of other small molecule kinase structures; however, as Lys53 is a highly conserved ATP-binding residue, this interaction is unlikely to contribute to selectivity. Whereas pyridine and imidazole serve as adenine mimetics important for affinity, the fluorophenyl group, which occupies a region of the active site not utilized by ATP, appears to be the key feature of the inhibitors contributing to selectivity. The corresponding key feature of the protein that appears to impart selectivity is Thr106, which forms one face of the fluorophenyl binding pocket. A larger side chain at this position blocks formation of this binding pocket. For example, ERK2, which has the larger glutamine side chain at position 106, is insensitive to SB 203580 (Z. L. Wang *et al.*, 1998). Because a limited number of kinases (10% of Ser/Thr kinases and 53% of Tyr kinases) (Hanks and Quinn, 1991) have side chains the size of threonine or smaller (Val or Ser), the relatively rare occurrence of the

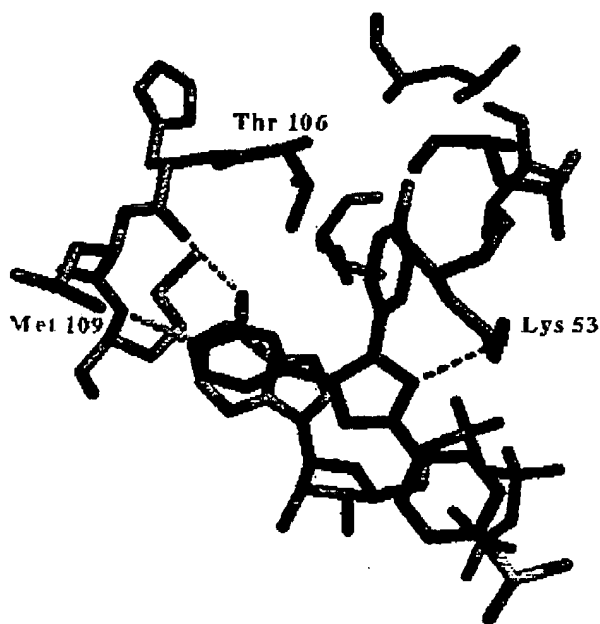


FIGURE 1. Active-site view of crystal structure for SB 203580 in p38. Protein residues shown include the hinge region T106 to M109 and residues K53, T106 which form the aryl binding pocket. Hydrogen bonds are shown as dotted lines. Protein kinase A coordinates for ATP have been used to position ATP.

fluorophenyl binding pocket appears to be an important feature governing selectivity of p38 kinase inhibitors.

8. PHARMACOLOGY OF PYRIDINYLMIDAZOLE p38 MITOGEN-ACTIVATED PROTEIN KINASE INHIBITORS

The p38 MAPK inhibitors described here are potent inhibitors of the synthesis of pro-inflammatory cytokines, such as IL-1 and TNF, *in vitro*. Many of these cytokines, when produced in excess or a dysregulated fashion, have been clearly shown to initiate and/or cause progression of inflammatory diseases (Lee *et al.*, 1988, 1994; Lee and Young, 1996).

SB 203580 has been profiled in a number of pharmacological models both *in vitro* and *in vivo* and demonstrated its activity in a wide variety of TNF- α -mediated animal models (Badger *et al.*, 1996). SB 203580 inhibits LPS-induced TNF- α *in vivo* in both mice and rats, with IC_{50} s of 15 and 25 mg/kg, respectively. SB 203580 dosed for 7 days at 50 mg/kg orally (b.i.d.) reduces joint edema in the collagen-induced arthritis model in DBA/1 LACJ mice. In addition, SB 203580 is effective in inhibiting IL-1-induced nitric oxide production in bovine articular cartilage explants and chondrocytes (Badger *et al.*, 1998). This reduction in nitric oxide production is a result of decreased transcription of the inducible nitric oxide synthase gene. SB 203580 is highly

effective in reducing paw inflammation in the adjuvant arthritic (AA) rat at doses of 30 and 60 mg/kg/day, with optimum inhibition observed at 60 mg/kg/day (86% inhibition on day 16). Evidence for protection of joint integrity is supported by a normalization of bone mineral density and bone content. Histological evaluation of the affected joints reveals a clear beneficial effect on both bone and cartilage. Serum levels of IL-6 are also reduced in drug-treated AA rats.

The protection of bone integrity in the AA rat led us to evaluate SB 203580 in a direct *in vitro* assay of bone resorption, the fetal rat long-bone assay. SB 203580 dose-dependently inhibits parathyroid hormone-stimulated bone resorption (IC_{50} , 0.6 μ M). Although the precise mechanism of action of the compound (and other pyridinylimidazoles) on bone resorption has not been fully defined, it appears to be related to the compound's cytokine suppressive properties, as selective cyclooxygenase and dual cyclooxygenase/lipoxygenase inhibitors were inactive in this organ culture system (Votta and Bertolini, 1994).

Another animal model where TNF- α has been shown to play an important role is that of endotoxin-induced shock. SB 203580 reduces serum TNF- α in LPS/D-gal sensitized mice and improves their survival at high doses (Badger *et al.*, 1996). While it is clear that SB 203580 is a potent inhibitor of IL-1 and TNF- α *in vitro* and that it is pharmacologically active in a number of animal models *in vivo*, the question arose whether such a potent cytokine inhibitor would also be immunosuppressive. This has been addressed by examining its activity *in vivo* in mice immunized with ovalbumin (OVA). Apart from partial inhibition of specific antibody levels against OVA, there is no suppression of OVA-specific T-cell proliferation, an allogeneic response, or of mitogen (concanavalin A)-induced proliferative responses (Badger *et al.* 1996). The pharmacological profile that has been described for SB 203580, a potent p38 MAPK inhibitor, would appear to be one that would be desirable for an anti-arthritis therapeutic agent.

9. CONCLUSION AND PERSPECTIVES

The recent discovery of several potent inhibitors of protein kinases that appear to retain considerable *in vitro* and *in vivo* specificity, despite competing at the ATP site, bodes well for the development of therapeutic agents directed towards blocking protein kinase activity. The pyridinylimidazole p38 MAPK inhibitors are supportive of this promise. Through the in-depth understanding of the mechanisms of action, it is possible to correlate structural aspects of inhibitor/kinase interactions at the molecular level. Further understanding of the basis for kinase specificity will come from an understanding of the detailed interactions between enzyme and inhibitors through mechanistic enzymology, structural, and mutagenesis studies. These insights have helped in the understanding of p38 MAPK inhibition. Availability of selective kinase inhibitors, even as ATP competitors, could provide invaluable tools to dissect the pathophysiological roles of protein kinases, and may even

help to identify suitable inhibitors for clinical use as novel therapeutics.

Acknowledgements—We thank Elizabeth Goldsmith of the University of Texas Southwest Medical Center for providing the SB 203580 p38 coordinates and Dr. Michael Bower of SmithKline Beecham for preparation of the figure.

References

- Adams, J. L., Boehm, J. C., Kassis, S., Corycki, P. D., Webb, E. F., Hall, R., Sorenson, M., Lee, J. C., Ayrton, A., Griswold, D. E. and Gallagher, T. F. (1998) Pyrimidinylimidazole inhibitors of CSBP/p38 kinase demonstrating decreased inhibition for hepatic cytochrome P450 enzymes. *Bioorg. Med. Chem. Lett.* 8: 3111–3116.
- Badger, A. M., Bradbeer, J. N., Votta, B., Lee, J. C., Adams, J. L. and Griswold, D. E. (1996) Pharmacological profile of SB 203580, a selective inhibitor of CSBP/p38 kinase, in animal models of arthritis, bone resorption, endotoxin shock and immune function. *J. Pharmacol. Exp. Ther.* 279: 1453–1461.
- Badger, A. M., Cook, M. N., Lark, M. W., Newnham-Tarr, T. M., Swift, R. A., Nelson, A. H., Barone, F. C. and Kumar, S. (1998) SB 203580 inhibits p38 mitogen-activated protein kinase, nitric oxide production, and inducible nitric oxide synthase in bovine cartilage-derived chondrocytes. *J. Immunol.* 161: 467–473.
- Ben-Levy, R., Hooper, S., Wilson, R., Paterson, H. F. and Marshall, C. J. (1998) Nuclear export of the stress-activated protein kinase p38 mediated its substrate MAPKAP kinase-2. *Curr. Biol.* 8: 1049–1057.
- Boehm, J. C., Smietana, J. M., Sorenson, M. E., Garigipati, R. S., Gallagher, T. F., Sheldrake, P. L., Bradbeer, J., Badger, A. M., Laydon, J. T., Lee, J. C., Hillegeass, L. M., Griswold, D. E., Breton, J. J., Chabonfletcher, M. C. and Adams, J. L. (1996) 1-Substituted 4-aryl-5-pyridinylimidazoles—a new class of cytokine suppressive drugs with low 5-lipoxygenase and cyclooxygenase inhibitory potency. *J. Med. Chem.* 39: 3929–3937.
- Boemer, R. J., Barker, S. C. and Knight, W. B. (1995) Kinetic mechanisms of the forward and reverse $p60^{src}$ tyrosine kinase reactions. *Biochemistry* 34: 16419–16423.
- Cobb, M. H. and Goldsmith, E. J. (1995) How MAP kinases are regulated. *J. Biol. Chem.* 270: 14843–14846.
- Cook, P. F., Neville, M. E., Jr., Vrana, K. E., Hartl, F. T. and Roskaski, R., Jr. (1982) Adenosine cyclic 3'-5'-monophosphate dependent protein kinase: kinetic mechanism for the bovine skeletal muscle catalytic subunit. *Biochemistry* 21: 5794–5799.
- Cuenda, A., Rouse, J., Doza, Y. N., Meier, R., Cohen, P., Gallagher, T. F., Young, P. R. and Lee, J. C. (1995) SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. *FEBS Lett.* 364: 229–233.
- Cuenda, A., Cohen, P., Bu'eo-Scherrer, V. and Goedert, M. (1997) Activation of stress-activated protein kinase-3 (SAPK3) by cytokines and other cellular stresses is mediated by SAPKK3 (MKK6), comparison of the specificities of SAPK3 and SAPK2 (RKKp38). *EMBO J.* 16: 295–305.
- Dean, M., Clifton, A. D., Lucocq, J. M. and Alessi, D. R. (1998) Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. *EMBO J.* 17: 4426–4441.
- de Lusszo, S. E., Visco, D., Agarwal, L., Chang, L., Chin, J., Croft, G., Forsyth, A., Fletcher, D., Frantz, B., Hacker, C., Hanlon,

- W., Harper, C., Kostura, M., Li, B., Luell, S., MacCoss, M., Mantlo, N., O'Neill, E. A., Orevillo, C., Pang, M., Parsons, J., Rolando, A., Sahly, Y., Sidler, K., Widmer, W. R. and O'Keefe, S. J. (1998) Pyrroles and other heterocycles as inhibitor of p38 kinase. *Bioorg. Med. Chem. Lett.* 8: 2689-2694.
- Derijard, B., Hib, I. M., Wu, I.-H., Barrett, T., Su, B., Deng, T., Karin, M. and Davis, R. J. (1994) JNK1: a protein kinase stimulated by UV light and H-Ras that binds and phosphorylates the c-jun activation domain. *Cell* 76: 1025-1037.
- Derijard, B., Raingeaud, J., Barrett, T., Wu, I. H., Han, J., Ulevitch, R. J. and Davis, R. J. (1995) Independent human MAP kinase signal transduction pathways defined by MEK and MKK isoforms. *Science* 267: 682-685.
- Enslin, H., Raingeaud, J. and Davis, R. J. (1998) Selective activation of p38 mitogen-activated protein (MAP) kinase isoforms by the MAP kinase kinases MKK3 and MKK6. *J. Biol. Chem.* 273: 1741-1748.
- Eyers, P. A., Craxton, M., Morrice, N., Cohen, P. and Goedert, M. (1998) Conversion of SB 203580-insensitive MAP kinase family members to drug-sensitive forms by a single amino-acid substitution. *Chem. Biol.* 5: 321-328.
- Frantz, B., Klatt, T., Pang, M., Parsons, J., Rolando, A., Williams, H., Tocci, M. J., O'Keefe, S. J. and O'Neill, E. A. (1998) The activation state of p38 mitogen-activated protein kinase determines the efficiency of ATP competition of pyridinylimidazole inhibitor binding. *Biochemistry* 37: 13846-13853.
- Freshney, N. W., Rawlinson, L., Guesdon, F., Jones, E., Cowley, S., Hsuan, J. and Saklatvala, J. (1994) Interleukin-1 activates a novel protein kinase cascade that results in the phosphorylation of Hsp27. *Cell* 78: 1039-1049.
- Fukunaga, R. and Hunter, T. (1997) MNK1: a new MAP kinase-activated protein kinase, isolated by a novel expression screening method for identifying protein kinase substrates. *EMBO J.* 16: 1921-1933.
- Gallagher, T. F., Seibel, G. L., Kassis, S., Laydon, J. T., Lee, J. C., Lee, D., Boehm, J. C., Bender, P. E., Erhard, K. F., Krog, A. J., Hofmann, G. A. and Adams, J. L. (1997) Regulation of stress-induced cytokine production by pyridinylimidazoles: inhibition of CSBP kinase. *Bioorg. Med. Chem.* 5: 49-64.
- Gille, H., Sharrocks, A. D. and Shaw, P. (1992) Phosphorylation of transcription factor p62TCF by MAP kinase stimulates ternary complex formation at c-fos promoter. *Nature* 358: 414-417.
- Goedert, M., Cuenda, A., Craxton, M., Jakes, R. and Cohen, P. (1997) Activation of the novel stress-activated protein kinase SAPK4 by cytokines and cellular stresses is mediated by SKK3 (MKK6)—comparison of its substrate specificity with that of other SAP kinases. *EMBO J.* 16: 3563-3571.
- Guni, R. J., McLaughlin, M. M., Kumar, S., Wang, Z. L., Bower, M. J., Lee, J. C., Adams, J. L., Livi, G. P., Goldsmith, E. J. and Young, P. R. (1998) Acquisition of sensitivity of stress-activated protein kinases to the p38 inhibitor, SB 203580, by alteration of one or more amino acids within the ATP binding pocket. *J. Biol. Chem.* 273: 15605-15610.
- Han, J., Lee, J.-D., Bibb, S. L. and Ulevitch, R. J. (1994) A MAP kinase targeted by endotoxin and in mammalian cells. *Science* 265: 808-811.
- Han, J., Lee, J.-D., Jiang, Y., Li, Z., Feng, L. and Ulevitch, R. J. (1996) Characterization of the structure and function of a novel MAP kinase kinase (MKK6). *J. Biol. Chem.* 271: 2886-2891.
- Han, J., Jiang, Y., Li, Z., Kravchenko, V. V. and Ulevitch, R. J. (1997) Activation of the transcription factor MEF2C by the MAP kinase p38 in inflammation. *Nature* 386: 296-299.
- Hanks, S. and Quinn, A. M. (1991) Protein kinase catalytic domain sequence database: identification of conserved features of primary structure and classification of family members. *Methods Enzymol.* 200: 38-62.
- Hanson, G. J. (1997) Inhibitors of p38 kinase. *Expert Opin. Ther. Patents* 7: 729-733.
- Henry, J. R., Rupert, K. C., Dodd, J. H., Turchi, I. J., Wadsworth, S. A., Cavender, D. E., Fahmy, B., Olini, G. C., Davis, J. E., Pellegrino-Gensey, J. L., Schafer, P. H. and Siskierka, J. J. (1998) 6-Amino-2-(4-fluorophenyl)-4-methoxy-3-(4-pyridyl)-1H-pyrrolo[2,3-b]pyridine (RW) 68354: a potent and selective p38 kinase inhibitor. *J. Med. Chem.* 41: 4196-4198.
- Jackson, J. R., Bolognese, B., Hillegass, L., Kassis, S., Adams, J., Griswold, D. E. and Winkler, J. D. (1998) Pharmacological effects of SB 220025, a selective inhibitor of p38 mitogen-activated protein kinase, in angiogenesis and chronic inflammatory disease models. *J. Pharmacol. Exp. Ther.* 284: 687-692.
- Jiang, Y., Gra, H., Zhao, M., New, L. G., Gu, J., Feng, L. L., Dipadova, F., Ulevitch, R. J. and Han, J. H. (1997) Characterization of the structure and function of the fourth member of p38 group of mitogen-activated protein kinases. *J. Biol. Chem.* 272: 30122-30128.
- Kracht, M., Shirao, M., Marshall, C. J., Hsuan, J. J. and Saklatvala, J. (1994) Interleukin-1 activates a novel protein kinase that phosphorylates the epidermal-growth-factor receptor peptide T669. *Biochem. J.* 302: 897-905.
- Kumar, S., McLaughlin, M. M., McDonnell, P. C., Lee, J. C., Livi, G. P. and Young, P. R. (1995) Human mitogen-activated protein kinase CSBP1, but not CSBP2, complements a *hog1* deletion in yeast. *J. Biol. Chem.* 270: 29043-29046.
- Kutnar, S., McDonnell, P. C., Gum, R. J., Hand, A. T., Lee, J. C. and Young, P. R. (1997) Novel homologues of CSBP/p38 MAP kinase: activation, substrate specificity and sensitivity to inhibition by pyridinyl imidazoles. *Biochem. Biophys. Res. Commun.* 235: 533-553.
- Kyriakis, J. M., Banerjee, P., Nikolakak, E., Dai, I. T., Rubi, E. A. E., Ahmad, M. F., Avruch, J. and Woodgett, J. R. (1994) The stress activated protein kinase subfamily of c-Jun kinases. *Nature* 369: 156-160.
- Lee, J. C. and Young, P. R. (1996) Role of CSBP/p38/RK stress response kinase in LPS and cytokine signaling mechanisms. *J. Leukocyte Biol.* 59: 152-157.
- Lee, J. C., Griswold, D. E., Votta, B. and Hanuma, N. (1988) Inhibition of monocyte IL-1 production by the anti-inflammatory compound, SK&F 86002. *Int. J. Immunopharmacol.* 10: 835-843.
- Lee, J. C., Rebar, L. and Laydon, J. T. (1989) Effect of SK&F 86002 on cytokine production by human monocytes. *Agents Actions* 27: 277-279.
- Lee, J. C., Votta, B., Dalron, B. J., Griswold, D. E., Bender, P. E. and Hanuma, N. (1990) Inhibition of human monocyte IL-1 production by SK&F 86002. *Int. J. Immunother.* VI: 1-12.
- Lee, J. C., Badger, A. M., Griswold, D. E., Dunnington, D., Truneh, A., Votta, B., White, J. R., Young, P. R. and Bender, P. E. (1993) Bicyclic imidazoles as a novel class of cytokine biosynthesis inhibitors. *Ann. NY Acad. Sci.* 696: 149-170.
- Lee, J. C., Laydon, J. T., McDonnell, P. C., Gallagher, T. F., Kumar, S., Green, D., McNulty, D., Blumenthal, M. J., Heys, J. R., Landvatter, S. W., Strickler, J. E., McLaughlin, M. M., Siemens, I. R., Fisher, S. M., Livi, G. P., White, J. R., Adams,

- J. L. and Young, P. R. (1994) A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* 372: 739-746.
- Li, Z. J., Jiang, Y., Ulevitch, R. J. and Han, J. H. (1996) The primary structure of p38-gamma—a new member of p38 group of MAP kinases. *Biochem. Biophys. Res. Commun.* 228: 334-340.
- Lin, A., Minden, A., Martinetto, H., Claret, F.-X., Lange-Carter, C., Mercurio, F., Johnson, G. L. and Karin, M. (1995) Identification of a dual specificity kinase that activates the Jun kinases and p38-Mpk2. *Science* 268: 286-290.
- LoGrasso, P. V., Frantz, B., Rolando, A. H., O'Keefe, S. J., Hermes, J. D. and O'Neill, E. A. (1997) Kinetic mechanism for p38 MAP kinase. *Biochemistry* 36: 10422-10427.
- McLaughlin, M. M., Kumar, S., McDonnell, P. C., Van Horn, S., Lee, J. C., Livi, G. P. and Young, P. R. (1996) Identification of MAPKAP kinase-3, a novel substrate of CSBP/p38 MAP kinase. *J. Biol. Chem.* 271: 8488-8492.
- Mertens, S., Craxton, M. and Goedert, M. (1996) SAP kinase-3, a new member of the family of mammalian stress-activated protein kinases. *FEBS Lett.* 383: 273-276.
- New, L., Jiang, Y., Zhao, M., Liu, K., Zhu, W., Flood, L. J., Kao, Y., Perry, G. C. N. and Han, J. H. (1998) PRAK, a novel protein kinase regulated by the p38 MAP kinase. *EMBO J.* 17: 3372-3384.
- Parker, C. G., Hunt, J., Diener, K., McGinley, M., Soriano, B., Kessler, G. A., Bray, J., Yao, Z. B., Wang, X. S., Kohn, T. and Lichenstein, H. S. (1998) Identification of stathmin as a novel substrate for p38 delta. *Biochem. Biophys. Res. Commun.* 249: 791-796.
- Posner, I., Engel, M. and Levitzki, A. (1992) Kinetic model of the epidermal growth factor (EGF) receptor tyrosine kinase and a possible mechanism of its activation by EGF. *J. Biol. Chem.* 267: 20638-20647.
- Preville, X., Schultz, H., Knauf, U., Gaestel, M. and Arrigo, A. P. (1998) Analysis of the role of Hsp25 phosphorylation reveals the importance of the oligomerization state of this small heat shock protein in its protective function against TNFalpha- and hydrogen peroxide-induced cell death. *J. Cell. Biochem.* 69: 436-452.
- Raingeaud, J., Gupta, S., Rogers, J. S., Dickens, M., Han, J., Ulevitch, R. J. and Davis, R. J. (1995) Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. *J. Biol. Chem.* 270: 7420-7426.
- Raingeaud, J., Whitmarsh, A. J., Barrett, T., Derijard, B. and Davis, R. J. (1996) MKK3- and MKK6-regulated gene expression is mediated by the p38 mitogen-activated protein kinase signal transduction pathway. *Mol. Cell. Biol.* 16: 1247-1255.
- Rouse, J., Cohen, P., Trigon, S., Morange, M., Alonso-Llamazares, A., Zanarillo, D., Hunt, T. and Nebreda, A. R. (1994) Identification of a novel protein kinase cascade stimulated by chemical stress and heat shock which activates MAP kinase-activated protein MAPKAP kinase-2 and induces phosphorylation of the small heat shock proteins. *Cell* 78: 1027-1037.
- Sanchez, I., Hughes, R. T., Mayer, B. J., Yee, K., Woodgett, J. R., Avruch, J., Kyriakis, J. M. and Zon, L. I. (1994) Role of SAPK/ERK kinase-1 in the stress-activated pathway regulating transcription factor c-jun. *Nature* 372: 794-798.
- Stein, B., Yang, M. X., Young, D. B., Janknecht, R., Hunter, T., Murray, B. W. and Barless, M. S. (1997) p38-2, a novel mitogen-activated protein kinase with distinct properties. *J. Biol. Chem.* 272: 19509-19517.
- Tong, L., Pav, S., White, D. H., Rogers, S., Crane, K. M., Cywin, C. L., Brown, M. L. and Pargellis, C. A. (1997) A highly specific inhibitor of human p38 HAP kinase binds in the ATP pocket. *Nature Struct. Biol.* 4: 311-316.
- Votta, B. J. and Bertolini, D. R. (1994) Cytokine suppressive anti-inflammatory compounds inhibit bone resorption in vitro. *Bone* 15: 533-538.
- Wang, X., Flynn, A., Waskiewicz, A. J., Webb, B. L., Vries, R. C., Baines, I. A., Cooper, J. A. and Proud, C. (1998) The phosphorylation of eukaryotic initiation factor eIF4E in response to phorbol esters, cell stresses, and cytokines is mediated by distinct MAP kinase pathways. *J. Biol. Chem.* 273: 9373-9377.
- Wang, X. Z. and Ron, D. (1996) Stress-induced phosphorylation and activation of the transcription factor CHOP (GADD153) by p38 HAP kinase. *Science* 272: 1347-1349.
- Wang, Z., Harkins, P. C., Ulevitch, R. J., Han, J., Cobb, M. H. and Goldsmith, E. J. (1997) The structure of mitogen activated protein kinase p38 at 2.1 Å resolution. *Proc. Natl. Acad. Sci.* 94: 2327-2332.
- Wang, Z. L., Canagarajah, B. J., Boehm, J. C., Kassis, S., Cobb, M. H., Young, P. R., Abdel-Meguid, S., Adams, J. L. and Goldsmith, E. J. (1998) Structural basis of inhibitor selectivity in MAP kinases. *Structure* 6: 1117-1128.
- Waskiewicz, A. J., Flynn, A., Proud, C. G. and Cooper, J. A. (1997) Mitogen-activated protein kinases activate the serine/threonine kinases MNK1 and MNK2. *EMBO J.* 16: 1909-1920.
- Wen, W., Meinkoth, J. L., Tsien, R. Y. and Taylor, S. S. (1995) Identification of a signal for rapid export of proteins from the nucleus. *Cell* 82: 463-473.
- Wilson, K. P., Fitzgibbon, M. J., Caron, P. N., Griffith, J. P., Chen, W. Y., McCaffrey, P. G., Chambers, S. P. and Su, M. S. (1996) Crystal structure of p38 mitogen-activated protein kinase. *J. Biol. Chem.* 271: 27696-27700.
- Wilson, K. P., McCaffrey, P. G., Iisano, K., Pazhanisamy, S., Galullo, V., Bemis, G. W., Fitzgibbon, M. J., Caron, P. R., Murcko, H. A. and Su, M. S. (1997) The structural basis for the specificity of pyridinylimidazole inhibitors of p38 MAP kinase. *Chem. Biol.* 4: 423-431.
- Young, P. R., McDonnell, P., Dunnington, D., Hand, A., Laydon, J. and Lee, J. C. (1993) Bicyclic imidazoles inhibit IL-1 and TNF production at the protein level. *Agents Actions* 39: C67-C69.
- Young, P. R., McLaughlin, M. M., Kumar, S., Kassis, S., Doyle, M. L., McNulty, D., Gallagher, T. F., Fisher, S., McDonnell, P. C., Carr, S. A., Huddleston, M. J., Seibel, G., Porter, T. G., Livi, G. P., Adams, J. L. and Lee, J. C. (1997) Pyridinyl imidazole inhibitors of p38 MAP kinase bind in the ATP site. *J. Biol. Chem.* 272: 12116-12121.